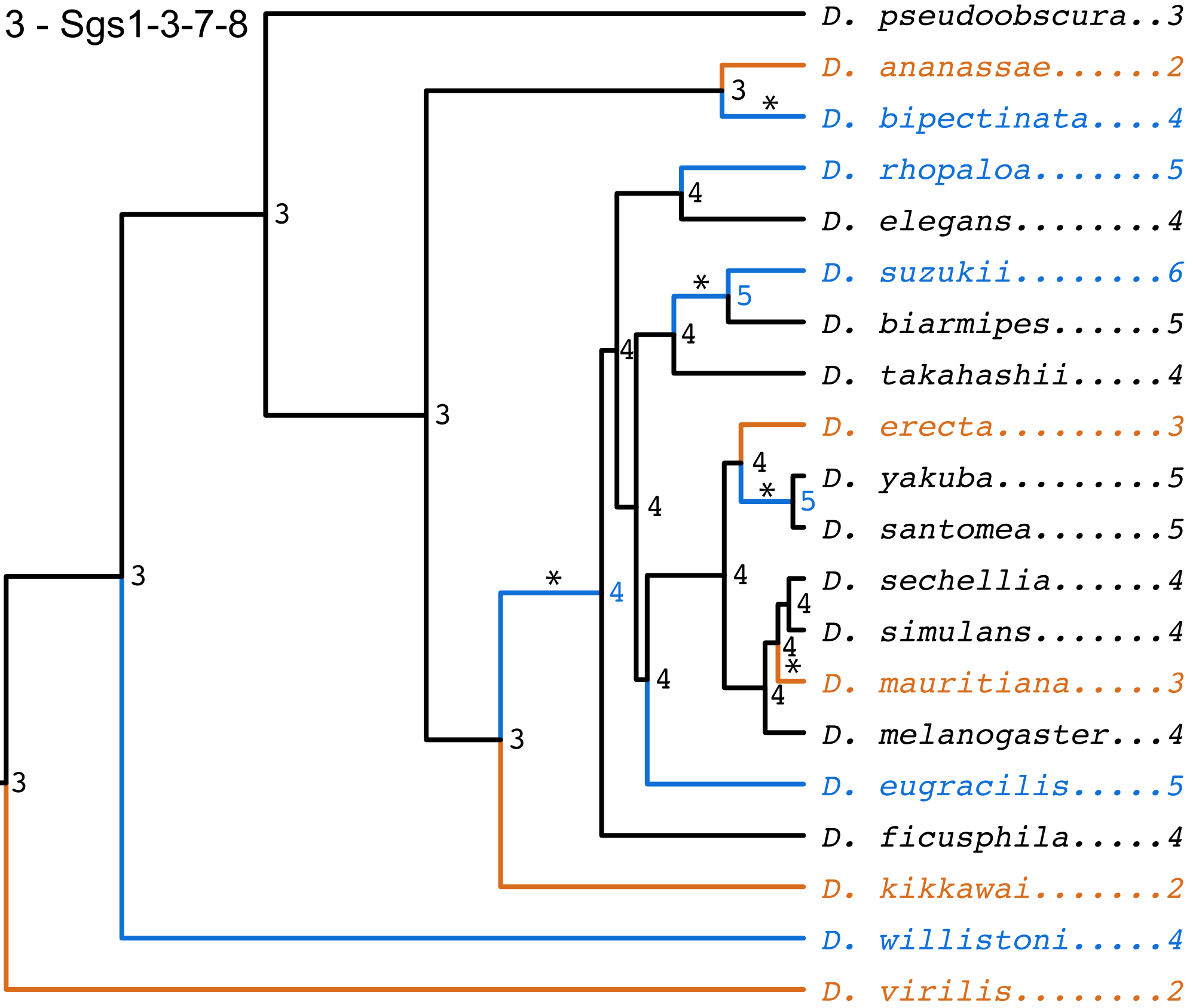
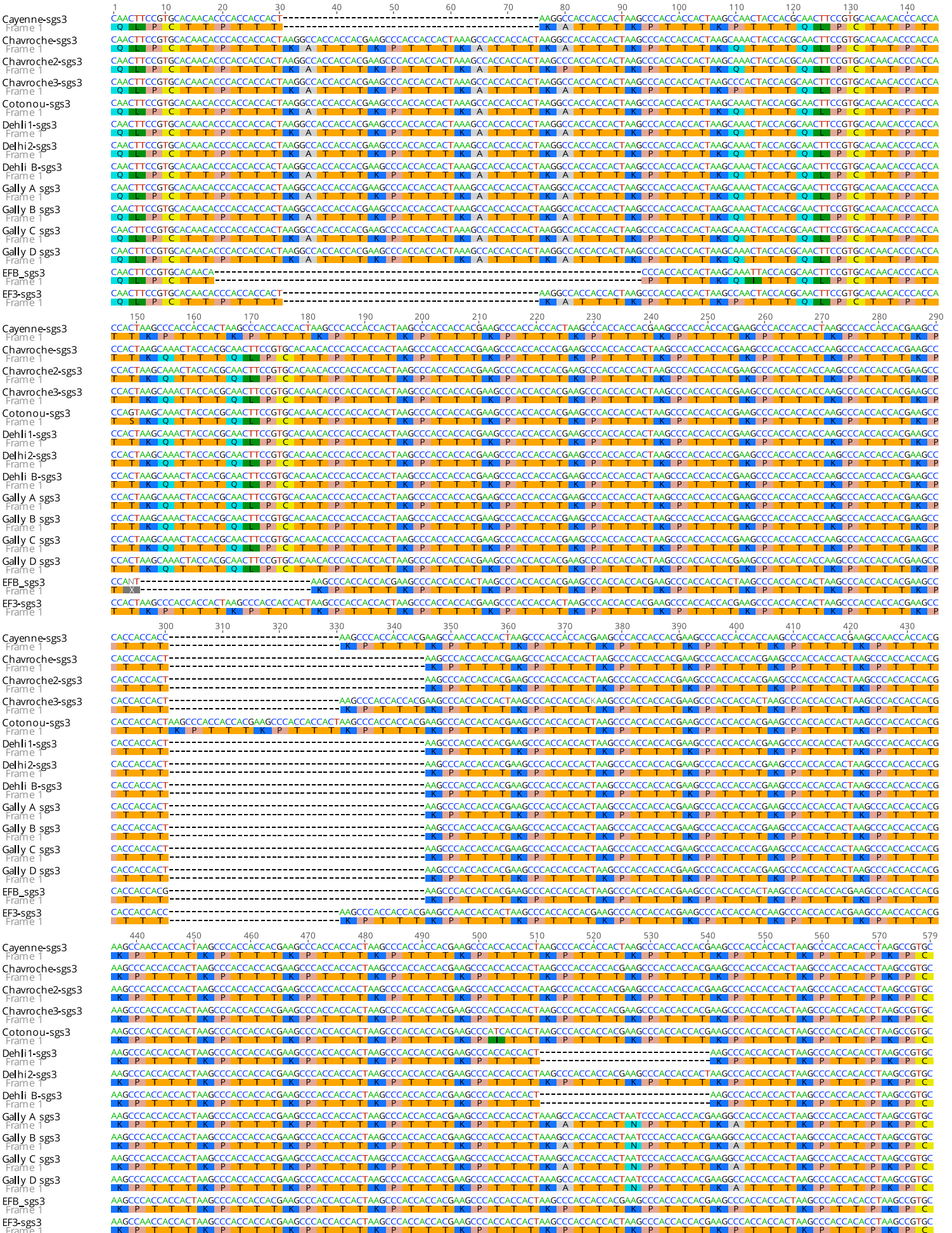
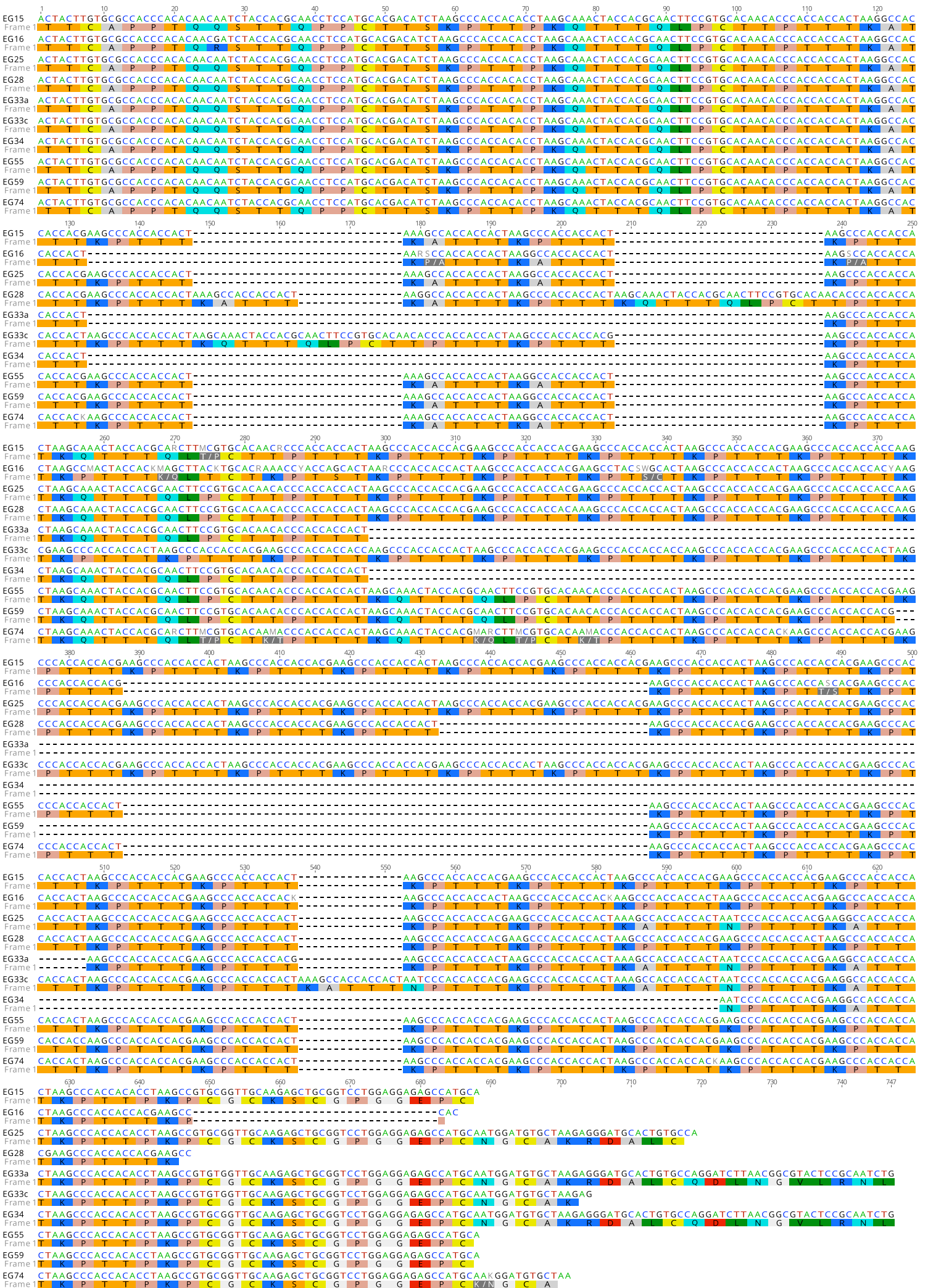


3 - Sgs1-3-7-8

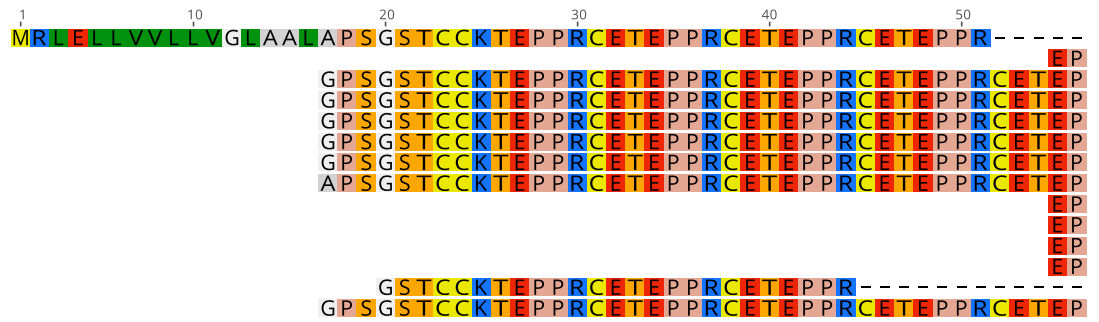




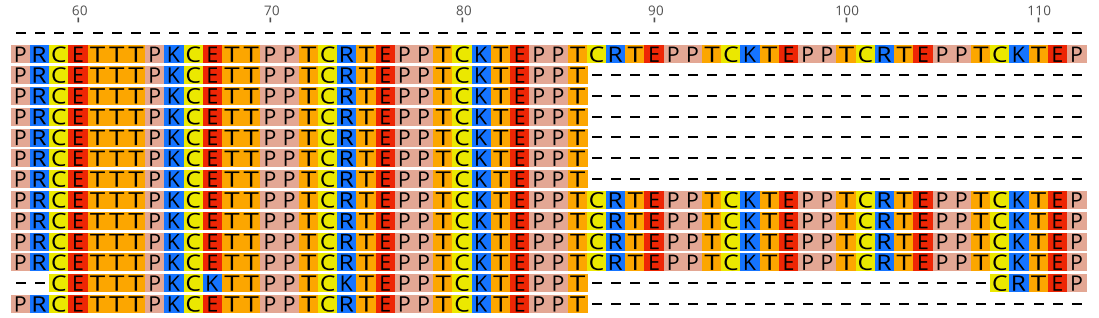


Genomic alignment tracks for various samples (e.g., FHD sggs4 CG1281, FHD Cayenne1\_sgs4, FHD Cayenne2\_sgs4, FHD Cayenne3\_sgs4, FHD Chvoroche1\_sgs4, FHD Chvoroche2\_sgs4, FHD Chvoroche3\_sgs4, FHD Gally1\_sgs4, FHD Gally2\_sgs4, FHD Delh1\_sgs4, FHD Delh2\_sgs4, FHD Odonou1\_sgs4, FHD Odonou2\_sgs4, FHD Comores1\_sgs4, FHD Comores2\_sgs4, FHD Tai1\_sgs4, FHD Tai2\_sgs4, FHD Canton1\_sgs4, FHD EF\_sgs4) across a genomic region from 1 to 531. The tracks show sequence alignments with color-coded markers (red, green, blue, yellow) indicating specific features or mutations. The top track (FHD sggs4 CG1281) includes a reference sequence with positions 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 531. The tracks are organized into groups, with each group containing multiple sample tracks. The alignment tracks show the sequence of each sample relative to the reference sequence, with color-coded markers indicating specific features or mutations. The top track (FHD sggs4 CG1281) includes a reference sequence with positions 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 531. The tracks are organized into groups, with each group containing multiple sample tracks. The alignment tracks show the sequence of each sample relative to the reference sequence, with color-coded markers indicating specific features or mutations.

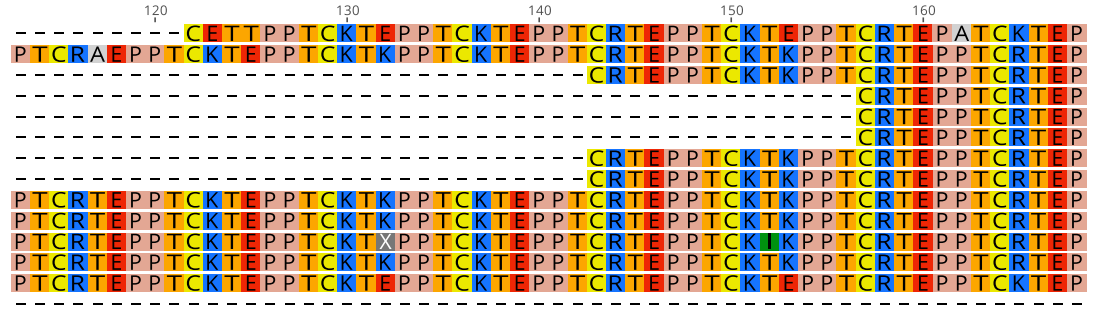
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sgs4\_EG16\_finrev  
sgs4\_EG25\_finrev  
sgs4\_EG28\_finrev  
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sgs4\_EG34\_finrev  
sgs4\_EG36\_finrev  
sgs4\_EG44\_finrev  
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sgs4\_ZI395\_finrev  
sgs4\_ZI420\_finrev



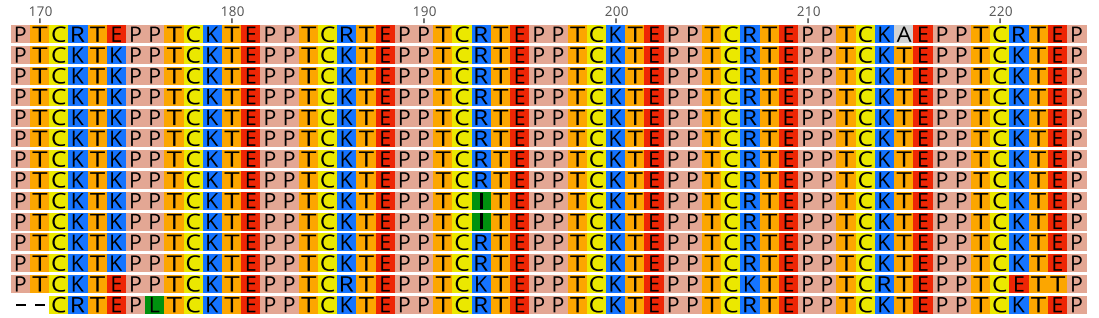
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sgs4\_EG36\_finrev  
sgs4\_EG44\_finrev  
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sgs4\_ZI420\_finrev



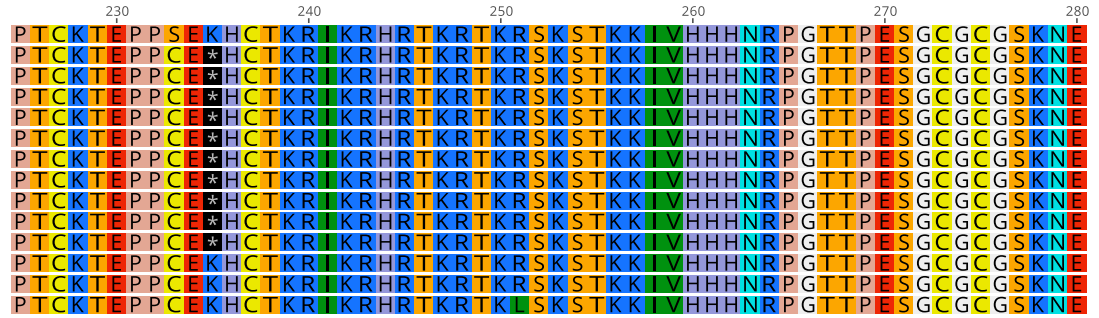
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sgs4\_ZI420\_finrev



sgs4-CG12181  
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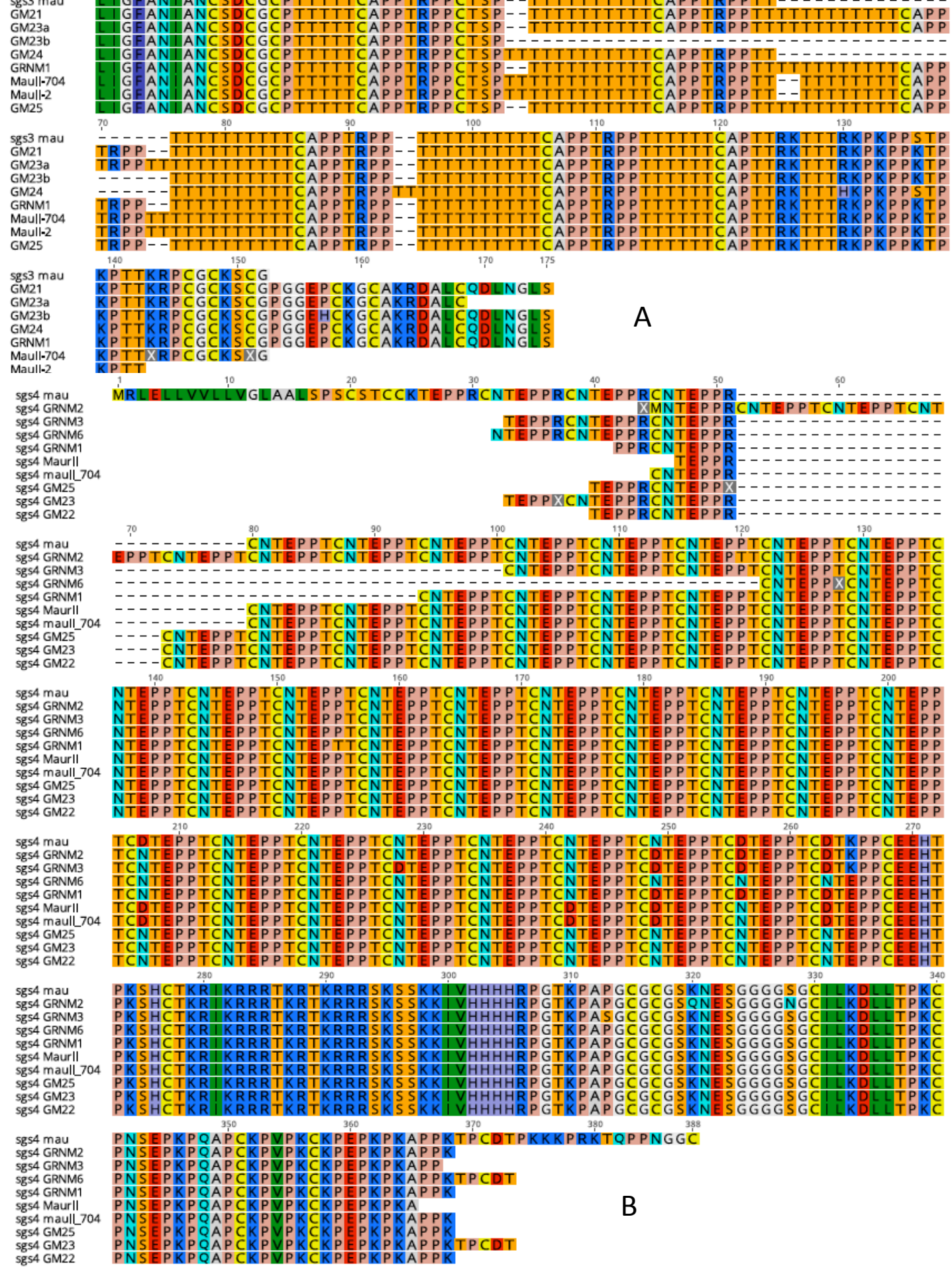


sgs4-CG12181  
sgs4\_EG15\_finrev  
sgs4\_EG16\_finrev  
sgs4\_EG25\_finrev  
sgs4\_EG28\_finrev  
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sgs4\_EG55\_finrev  
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sgs4\_ZI395\_finrev  
sgs4\_ZI420\_finrev



sgs4-CG12181  
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sgs4\_EG16\_finrev  
sgs4\_EG25\_finrev  
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sgs4\_EG33\_finrev  
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sgs4\_EG36\_finrev  
sgs4\_EG44\_finrev  
sgs4\_EG55\_finrev  
sgs4\_EG59\_finrev  
sgs4\_EG74\_finrev  
sgs4\_ZI395\_finrev  
sgs4\_ZI420\_finrev





	Feuille1-lam0.003				
	Sgs1	Sgs3-7-8 family	Sgs1-3-7-8 family	Sgs4	Sgs5-5bis family
D. melanogaster	1	3	4	1	2
D. simulans	1	3	4	1	2
D. sechellia	1	3	4	1	2
D. mauritiana	1	2	3	1	1
D. santomea	1	4	5	1	2
D. yakuba	1	4	5	1	2
D. erecta	0	3	3	1	1
D. eugracilis	1	4	5	0	2
D. takahashi	1	3	4	0	2
D. suzukii	1	5	6	0	2
D. biarmipes	1	4	5	0	2
D. elegans	1	3	4	0	1
D. rhopaloa	1	4	5	0	1
D. ficusphila	1	3	4	0	2
D. kikkawai	0	2	2	0	2
D. ananassae	0	2	2	0	2
D. bipectinata	0	4	4	0	2
D. pseudoobscura	0	3	3	0	1
D. willistoni	0	4	4	0	0
D. virilis	0	2	2	0	1
<b>Lambda value</b>	0,0036503	0,003650259	0,003650259	0,0036503	0,003650259
<b>P-value</b>	0,2925	0,013	0,005	0,5795	0,107
<b>Tree used</b>	(((drho:11.1(((drho:11.134(((drho:11.1347,((dere:5.733(((drho:11.1347				

7,dele:11.1347):5.86695,(((dsuz:6.86108,dbia:6.86108):4.95065,dtak:11.8117):3.4



2837,(((dere:5.73328,(dyak:1.0,dsan:1.0):4.73328):1.52117,(((dsec:1.35937,dsim:1.35937):

:1.0,dmau:2.35937):1.16816,dmel:3.52753):3.72691):7.01828,deug:14.24007):1.0):1.7615)

:1.39134,dfic:18.3929):9.20704;

**Table S2** : List of primers used for this study. Different combinations were used to amplify glue genes. All primers were chosen outside the repeated regions. *D. sechellia*, *D. santomea*, *D. virilis* and *D. biarmipes* were resequenced because of uncertainties or putative errors in the online sequences. *D. melanogaster* and *D. mauritiana* were resequenced for studying RNV in *Sgs3* and *Sgs4*, or confirming the presence of premature stop codons in *Sgs4* and *Sgs5* of *D. melanogaster*.

### ***Sgs1***

species	Forward primers	Reverse primers
<i>D. melanogaster</i>	ATGAAAGTCGCCCTTATCTT	TGTCAGTGTACAGGGTTGC
<i>D. sechellia</i>	TCGTCCTTGTCTTGCCATC	TTACGTTTCGTGTGCACAAGCA

### ***Sgs3***

species	Forward primers	Reverse primers
<i>D. melanogaster</i>	TCCGCTAATGTTGCCAACTG TAATGTTGCCAACTGTTGC CATGAAGCTGACCATTGCTA	CATCCATTGCATGGCTCTCC ATTGACGGATCTTGCGCTC GACGCATTGACGGATCTTGC
<i>D. sechellia</i>	GCTGACCATTGCTACCATTG	CTTAGCGCATCCTGTGCAAG
<i>D. santomea</i>	GAAGCTGACCATTGCCATCGC GTGCACAGTCCCTGCCTCTT	TAAGATCCGTGCACAGTCCC
<i>D. mauritiana</i>	ATCCTGCTTATTGGCTTCGC	AAGCCGTTGAGATCCTGGCA
<i>D. virilis</i>	TGCTCGGCTGAATGCGAGTG	CAAGAGCTCCTTGACAGAGCG GGTCCTTGTGGTGGACACGC
<i>D. biarmipes</i>	AGCCCATGTGAAGTGGAGTC GGATGCACCACCGGGACTGA ACCGGGACTGAATGTGGTTG TGGAGTCTCCACCCTGTGA	GGGCTCTCCGACGACGCATTG CATTGCCGGATCTTGCGTTC ACAAACGCACTGGCGGATTG TCCAGACCTCGGACTGTGTTG

### ***Sgs4***

species	Forward primers	Reverse primers
<i>D. melanogaster</i>	CACTTGCCCCGTCAGGTTCTAC TGTCTATGTCTAACCATCGC	TACAGCATGTAGAACCTGAC GCGGTTTCTTGGTGGTGTTC TGTCGCTTGATGCGTTTCGT
<i>D. sechellia</i>	GGTCTGGCTGCACTTTCGCC	TACAGCATGTAGAACCTGAC
<i>D. santomea</i>	TTATTGGTCGGGTTGGCTG TCGGGTTGGCTGCACTTAC	GGTGGTGGCACATGGTTTCG CACATGGTTTCGGCTGCGA
<i>D. mauritiana</i>	CTGCACTTTCGCCGTCATGT	CTGGGTCTTCGGTGGTTTC

### ***Sgs5***

species	Forward primers	Reverse primers
<i>D. melanogaster</i>	CATGGACAAGCCGTCAGGA	GTAACGTGATCCAACCTGAATCC

**Table S3:** Assembly/Annotation error estimation and gene gain/loss rates in a single  $\lambda$  model in the 25 *Drosophila* species included in this study compared to previous studies using fewer species.

	$\lambda$ (No Error Model)	$\epsilon$ (Estimated error)	$\lambda$ (Error Model = $\epsilon$ )
25 <i>Drosophila</i> species in this study	0.00365	0.04434	0.00340
12 <i>Drosophila</i> species (Han et al. 2013)	0.00121	0.04102	0.00059
12 <i>Drosophila</i> species (Hahn et al. 2007)	0.00121	NA	NA

**Table S4:** Summary of gene gain and loss events inferred after correcting for annotation and assembly error across all 25 *Drosophila* species. The number of rapidly evolving families is shown in parentheses for each type of change.

Species	Expansions			Contractions			No Change	Avg. Expansion
	Families	Genes gained	genes/expansion	Families	Genes lost	genes/contraction		
<i>pseudoobscura</i>	307 (55)	390	1.27	325 (79)	356	1.1	8747	0.003625
<i>miranda</i>	161 (14)	189	1.17	377 (27)	428	1.14	8841	-0.025482
<i>bipectinata</i>	425 (43)	579	1.36	167 (12)	206	1.23	8787	0.039770
<i>eugracilis</i>	461 (34)	649	1.41	271 (6)	311	1.15	8647	0.036038
<i>busckii</i>	844 (23)	1288	1.53	712 (5)	801	1.13	7823	0.051925
<i>takahashii</i>	477 (60)	874	1.83	139 (4)	148	1.06	8763	0.077407
<i>erecta</i>	102 (20)	132	1.29	198 (25)	286	1.44	9079	-0.016420
<i>sechellia</i>	1184 (233)	2020	1.71	443 (83)	480	1.08	7752	0.164197
<i>persimilis</i>	1243 (254)	1979	1.59	458 (66)	493	1.08	7678	0.158439
<i>kikkawai</i>	558 (30)	811	1.45	362 (4)	410	1.13	8459	0.042755
<i>arizonae</i>	154 (31)	163	1.06	531 (106)	578	1.09	8694	-0.044248
<i>virilis</i>	329 (21)	541	1.64	288 (1)	303	1.05	8762	0.025376
<i>elegans</i>	347 (24)	464	1.34	259 (24)	357	1.38	8773	0.011408
<i>suzukii</i>	1472 (105)	2108	1.43	219 (8)	238	1.09	7688	0.199382
<i>simulans</i>	151 (35)	192	1.27	168 (66)	260	1.55	9060	-0.007250
<i>yakuba</i>	673 (84)	891	1.32	120 (6)	145	1.21	8586	0.079539
<i>melanogaster</i>	60 (13)	88	1.47	166 (13)	196	1.18	9153	-0.011515
<i>mojavensis</i>	320 (101)	470	1.47	166 (47)	179	1.08	8893	0.031027
<i>grimshawi</i>	843 (25)	1218	1.44	633 (2)	683	1.08	7903	0.057042
<i>willistoni</i>	603 (13)	895	1.48	649 (1)	713	1.1	8127	0.019405
<i>navojoa</i>	202 (25)	227	1.12	1934 (74)	2200	1.14	7243	-0.210364
<i>rhopaloea</i>	1127 (96)	1753	1.56	153 (2)	159	1.04	8099	0.169954
<i>biarmipes</i>	150 (9)	184	1.23	230 (21)	288	1.25	8999	-0.011089
<i>ananassae</i>	270 (28)	464	1.72	237 (13)	278	1.17	8872	0.019832
<i>ficuspila</i>	514 (29)	685	1.33	341 (7)	393	1.15	8524	0.031133